Exhibit II

Extracellular Related Kinase Increases Adipocyte Lipolysis by Phosphorylating and Activating Hormone-Sensitive Lipase. Wen-Jun Shen, Kizito Muliro, Sandra C Souza, Melanie Halbleib, Fredric B. Kraemer., Andrew S. Greenberg, Palo Alto, CA, Boston, MA.

Hormonally stimulated lipolysis occurs by activation of cyclic-AMP dependent protein kinase (PKA) which phosphorylates hormone-sensitive lipase (HSL) and increases adipocyte lipolysis. Previously, we showed that treatment of 3T3-L1 adipocytes with either forskolin or catecholamines activates PKA and extracellular related kinase (ERK), and that incubation with PD 98059 (100 μM), a specific inhibitor of the upstream ERK activator,MEK-1,2, blocks both catecholamine and forskolin stimulated lipolysis by ~50%, supporting a role for ERK in lipolysis. We now demonstrate that two different MEK-1 inhibitors block catecholamine and CL316,243 (5μM), a beta-3 agonist, stimulated lipolysis by 50%. Furthermore, treatment of 3T3-L1 adipocytes with 1,2-dioctanoyl-sn-glycerol(50μM)(DAG,(a PKC analogue) activates ERK and increases lipolysis, while two different MEK-1,2 inhibitors decrease both basal (untreated) and DAG stimulated activation of ERK and lipolysis. When partially purified HSL was incubated with activated ERK and γ^{32}ATP in vitro, HSL was phosphorylated. To further study the regulation of HSL by ERK, we used a tamoxifen regulatable Raf system (ER-Raf) expressed in 3T3-L1 preadipocytes. When ER-Raf cells were transfected with HSL and exposed to tamoxifen (1 μM), ERK activation was seen in 15-30 min and HSL activity increased by ~ 2 fold. Putative ERK consensus phosphorylation sites were identified at Thr 540, Ser 600, Thr 614 and Ser 680. Mutation of Ser 600 to Ala eliminated the ability of HSL to be phosphorylated by active ERK in vitro. When S600A HSL was expressed in ER-Raf cells, tamoxifen treatment failed to increase its activity. Thus, activation of the ERK pathway appears to able to regulate adipocyte lipolysis by phosphorylating HSL on Ser 600 and increasing the activity of HSL.

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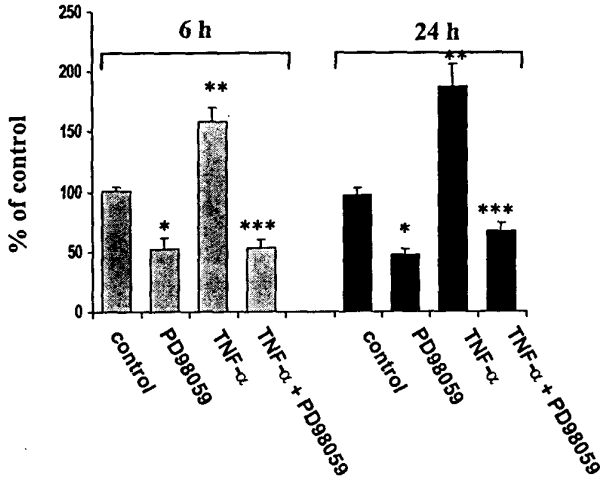
6 h
24 h
% of control
250
200
150
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control
PD98059
TNF-α
TNF-α + PD98059